REMARKS

Status

Claims 1-21 are pending in this application, no claims being added, amended or canceled herein.

Claims 18-21 were rejected under the judicially created doctrine of obviousness type double patenting as allegedly obvious over claims 1-62 of U.S. Patent No. 6,011,148. Claims 14-16 were rejected under the 35 U.S.C. §112, second paragraph on the grounds that they are indefinite. Claims 1-21 were rejected under 35 U.S.C. §103(a) as allegedly obvious over EP 517,515 A2 in view of U.S. Patent No. 6,197,553 B1, U.S. Patent No. 5,837,529 and Song *et al., J. Chem. Soc. Faraday Trans.* 91:3389-3398 (1995). Applicants respectfully traverse these rejections.

Obviousness-type double patenting:

Claims 18-21 were provisionally rejected under the judicially created doctrine of obviousness type double patenting as allegedly unpatentable over claims 1-62 of U.S. Patent No. 6,011,148. Upon notification of allowable subject matter, Applicants will file a terminal disclaimer, disclaiming any term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. §§ 154-156 and § 173 of prior Patent No. 6,011,148 issued January 4, 2000, thereby obviating the obviousness-type double patenting rejection.

35 U.S.C. §112, second paragraph, indefiniteness:

Claims 14-16 are rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite. In particular, the Examiner states that it is not clear from the claims or the text of the specification how one of skill in the art would carry out a mixing step in a flow through mixer, a centrifugation step and a neutralization step simultaneously. The Examiner states that each step would require a separate piece of equipment which would

be difficult to combine for the simultaneous performance of all three steps. Applicants respectfully disagree.

Figure 1 of the above referenced application is a line diagram of the invention showing that all elements of the plasmid purification process are connected by a series of tubes connected to buffer tanks and apparati so that the entire process can be carried out simultaneously. The specification describes the process beginning on page 6, line 20 and continuing to page 9, line 9. In brief, the cells to be lysed in tank 10 pass through line 20 and mix with the lysis mixture from tank 40. The cell/lysis mix passes through the static mixer 1, and precipitation solution is then added to the mixed solution. The mixed solution is then further mixed in static mixer 2. The resulting solution may be buffered prior to or after passing through a disk stack or decanting centrifuge. The specification states on page 9, lines 3-8:

> Appropriate sizing of the static mixers, pumps, and centrifuge, and selection of flow rate will allow for continuous operation of the process, while maximizing yield and quality. Preferably, the process is automated to ensure reproducibility. An example of conditions allowing continuous operation is described in Example 4 below.

Example 4 beginning on page 22, line 20 through page 23, line 11 and Table 3 recites conditions of flow rate, mixer pipe internal diameters, Reynolds, velocity, # of elements etc. to simultaneously perform the all of the steps of the purification process on a batch of cells. While one portion of the batch of cells is being lysed and passing through static mixer 1, a second portion of the batch of cells is mixing with the precipitation solution in static mixer 2 and a third portion of the batch of cells is spinning in the centrifuge. The specification states on page 23, lines 6-9, "[t]he flow rate is selected to match that desired for centrifugation, while still maintaining high plasmid DNA yield and minimizing genomic DNA contamination. This allows the lysis, precipitation and clarification steps to be performed simultaneously, without interruption, and to be automated."

Applicants submit that in view of the above remarks, the specification does conclude with claims which particularly point out and distinctly claim the subject matter which applicants regard as their invention and respectfully request that the §112, second paragraph rejection of claims 14-16 be withdrawn.

35 U.S.C. § 103(a):

Claims 1-21 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over EP 517,515 A2 ("Ogawa") in view of U.S. Patent No. 6,197,553 B1 ("Lee") U.S. Patent No. 5,837,529 ("Wan") and Song *et al., J. Chem. Soc. Faraday Trans.* 91:3389-3398 (1995) ("Song"). In particular, the Examiner alleges that Ogawa teaches a method of producing large quantities of plasmid DNA for pharmaceutical use by mixing the DNA and an alkali lysing agent, then adding a precipitating agent, removing the precipitated component by filtration followed by an ultrafiltration step. Ogawa discusses treating the samples with RNAse digestion and using potassium acetate. The Examiner admits that Ogawa does not teach a centrifugation step, a neutralization step or an ion exchange step.

The Examiner alleges that Lee teaches a method for purifying large quantities of plasmid DNA for pharmaceutical use by first heat lysing the cell mass in a flow through heat exchanger, followed by a centrifugation step, followed by a filtration step, followed by an ultrafiltration step, followed by an ion exchange step. The Examiner states that the flow rates cited in the claims are merely optimizations and are not patentably distinct.

The Examiner alleges that Wan teaches a method for purifying large quantities of plasmid DNA for pharmaceutical use by mixing the DNA and an alkaline lysis agent in a static mixer, and adding a precipitation agent in a second static mixer.

The Examiner alleges that Song teaches the general theory of concentration polarization on a membrane during ultrafiltration. Specifically, the Examiner states that Song teaches the process of ultrafiltration involves the development

of a polarization layer. As acknowledged by the Examiner, this layer provides resistance to flow through the ultrafilter.

The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of purifying large quantities of plasmid DNA of Ogawa with the method of purifying large quantities of DNA for pharmaceutical use of Lee and Wan because they all involve the process of purifying large quantities of DNA. Song provides the theoretical background for forming a gel layer. The Examiner states that one would be motivated to combine the method of Ogawa, Lee and Wan because Ogawa teaches that alkaline lysis may be used as an equivalent to heat lysis. The Examiner further states that Lee describes the need to produce large quantities of polynucleotide in purified form and that preparative scale chromatography is a powerful tool to purify plasmid DNA. The Examiner also states that Wan teaches that static mixers provide a distinct advantage for lysing large quantities of bacteria and that Song provides the theoretical background for the formation of a gel layer. Finally, the Examiner states that on of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention given the teachings of Ogawa, Lee, Wan and Song. Applicants respectfully disagree.

The Examiner is respectfully reminded that in order to find an invention *prima facie* obvious, the cited art must (1) teach or suggest each of the elements of the claimed invention, (2) provide suggestion or motivation to combine or modify the references, and (3) provide a reasonable expectation that one could successfully arrive at the claimed invention. See M.P.E.P. § 2143 *et seq.* Applicants submit that the Examiner has failed to make his *prima facie* case because the references in combination do not teach each and every element of the claimed invention, there is no suggestion or motivation to combine the references cited and there is no reasonable expectation of successfully arriving at the claimed invention.

The basic elements of the invention are defined in claim 1 as follows:

> A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:

a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;

b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;

c) contacting the lysed cell solution with a precipitation solution;

d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;

e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and

f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);

g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.

Claims 18-21 add the additional element of ultrafiltration through a gel layer.

The Examiner recognizes that the claims are drawn to a method of purifying at least 100 mg of plasmid DNA by mixing the cells with a lysis solution in a static mixer, adding a precipitating solution in a second static mixer, removing the precipitate by centrifugation, neutralizing the solution and passing the solution over an ion exchange resin. The prior art cited by the Examiner neither teaches nor suggests the neutralization step required by claim 1, step (f). Applicants teach adding a neutralizing agent to either the precipitation solution prior to centrifugation or to the resulting clarified solution following centrifugation (see step (f) of claim 1) to help preserve the integrity of the plasmid DNA during the purification process (see specification, page 8, lines 3-6).

The Examiner admits on page 4, second to last paragraph, that Ogawa does not teach the neutralization step. Ogawa teaches alkali lysis followed by a precipitation step, a filtration step and an ultrafiltration step (see Ogawa, column 4, lines 8-18). The deficiency in the teachings of Ogawa is not found in the other references cited by the Examiner. Lee does not teach the neutralization step. Lee teaches heat lysis in a

heat exchanger, followed by centrifugation, filtration, ultrafiltration and ion exchange chromatography (see Lee, column 5, lines 6-47 and claim 1). Wan also does not teach the neutralization step. Wan teaches alkali lysis in a static mixer followed by precipitation in a static mixer (see Wan, column 4, lines 15-28). Song describes the theoretical basis of gel layers.

Applicants submit that the Examiner has not established a *prima facie* case of obviousness because the Examiner has not cited references which teach all of the elements of the claimed invention. Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of claims 1-21 be withdrawn.

Assuming *arguendo* that the cited art did teach or suggest all of the elements of the claimed invention, the Examiner has failed to make his *prima facie* case of obviousness because there is no suggestion or motivation to combine or modify the references cited in a manner indicated by the Examiner. As stated by the Court of Appeals for the Federal Circuit:

> Our case law makes it clear that the best defense against hindsight-based obviousness analysis is the rigorous application of the requirement for a showing of a teaching or motivation to combine the prior art references. See *Dembiczak,* 175 F.3d at 999, 50 USPQ2d at 1617. "**Combining prior art references without evidence of such suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability--the essence of hindsight.**" **Id** [emphasis added] *Ecolochem, Inc. v Southern-California Edison Co.,* 227 F.3d 1361, 1371 (Fed. Cir. 2000)
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> The mere fact that the prior art may be modified in the manner suggested by the Examiner **does not** make the modification obvious unless the prior art suggested the desirability of the modification. [emphasis added] *In re Fritch*, 23 USPQ2d 1780, 1783-1784 (Fed. Cir. 1992)

As stated *supra* the presently pending claims consist of a unique combination of steps for the purification of pharmaceutical grade plasmid DNA that is not found in the cited references. The Examiner states that one of skill in the art would

have been motivated to combine the method of purifying large quantities of plasmid DNA of Ogawa with the large scale methods taught by Lee and Wan because Ogawa teaches that alkaline lysis is equivalent to heat lysis, Lee teaches preparative scale chromatography is a powerful purification tool to purify the various forms of plasmid DNA from host contaminants, Wan teaches that static mixers provide a distinct advantage to lysing bacterial cells and Song provides the theoretical background on the formation of a gel layer. Applicants respectfully disagree.

There is no suggestion or motivation in the prior art cited by the Examiner to combine or modify the art in the manner suggested by the Examiner. Ogawa, Lee and Wan each teach a method of purifying plasmid DNA from cells and each with different and unique steps for accomplishing that task. Ogawa, as described in examples 1 and 2 in column 4, lines 1-35, is directed to laboratory scale plasmid purification. Ogawa does not teach or suggest that this purification protocol can be scaled up to process the volume of cells required to isolate 100 mg of plasmid DNA as claimed.

Ogawa does not teach that the various lysis methods available to those of skill in the art are equivalent. Ogawa states in column 2, lines 13-14 that "[t]he cells may be lysed by any conventional method." Ogawa does not state that once lysed, the cell solutions are treated the same. In the instant Office Action, the Examiner has not provided any additional guidance for where Ogawa teaches that heat and alkali lysis protocols are equivalent.

Lee actually teaches away from the teachings of Ogawa and Wan in several respects. Lee teaches that the techniques used to purify plasmid DNA on the laboratory scale are not suitable to purify DNA on a large scale. Lee specifically teaches that alkaline lysis is not suitable for large scale plasmid purification. See Lee, column 1, lines 14-20 and Lee, column 1, lines 36-56. Lee suggests that other small or laboratory scale plasmid purification schemes are not suitable for high volume plasmid purification (see Lee, column 2, lines 3-20) and states new technology is required to be able to use DNA as a biopharmaceutical (see Lee, column 1, lines 43-46).

Ogawa, Lee and Wan fail to provide the motivation to combine their disclosed plasmid purification system with the plasmid purification methods disclosed by the others because each teaches that their method produces suitable plasmid DNA. Ogawa states in column 4, lines 53-54 that "the DNAs purified by the method of the present invention had high purity." This indicates that additional purification steps are not necessary. Lee also indicates that using their method, additional purification steps are not required (see example 4, Figures 8 and 9, and specifically column 9, lines 1-9). Wan discloses a plasmid purification method which combines an alkaline lysis step and a precipitation step in static mixers (see Wan, column 4, lines 7-28). Wan does not teach or suggest any additional purification steps.

Furthermore, Ogawa, Lee and Wan are silent about the benefits of a gel layer during the ultrafiltration step. Ogawa does not teach or suggest that a gel layer is necessary or desirable. Lee is also silent about the need of a gel layer. Lee discloses that diafiltering a plasmid sample is useful (see column 5, lines 35-47 and column 6, lines 16-28) but does not teach or suggest the need or desire to form a gel layer during the diafiltration process. Lee specifically does not disclose the steps disclosed in the above referenced application to insure gel layer formation (see specification for example page 13, line 22 through page 14, line 20). Wan specifically does not teach or suggest the use of any chromatography, anion exchange or ultrafiltration steps.

The Examiner relies on Song to teach the benefits of filtering through a gel layer during the ultrafiltration step. The teachings of Song are inapplicable to the claimed invention. The Examiner states that Song provides the theoretical basis for the formation of gel layers and that gel layers are inherent in all crossflow ultrafiltration processes. The Examiner is reminded, "[a] retrospective view of inherency is no substitute for some teaching or suggestion which supports the selection and use of the various elements in the particular claimed combination." *In re Newell* 891 F.2d 899 (Fed. Cir. 1989).

Song does not provide any teaching or suggestion supporting the benefits of a gel layer in the ultrafiltration process for plasmid DNA preparations. Song is a

theoretical paper explaining, by mathematical formulae, how gel layers develop and how flow rates through a membrane are affected by a gel layer or a cake layer which develops during an ultrafiltration process. Song's theory was developed based on "uniform non-interacting ("hard") spherical particles" (see Song, page 3389, column 2, last paragraph of the introduction). Important variables in the development of a gel layer which retards permeate flow across the membrane are discussed and the relationship of these variables to one another is summarized in Figure 4 page 3396. Methods to maximize permeate flux across the ultrafiltration membrane are also discussed. Song does not teach any conditions which would be suitable for plasmid DNA purification. More importantly, Song teaches that the object of the instant application, forming gel layers to aid purification of biological macromolecules should be minimized or controlled.

Song teaches away from the desirablility of having a gel layer because it provides a resistance to flow through the ultrafilter as acknowledged by the Examiner.

Song states:

> Concentration polarization is a phenomenon in which the solute or particle concentration in the vicinity of the membrane surface is higher than that in the bulk. This phenomenon, inherent to all crossflow filtration processes, occurs as long as the membrane shows different permeability for the various components of the solution or suspension. **The resulting concentrated layer at the membrane surface increases the filter resistance and consequently reduces the permeate flux through the membrane. Concentration polarization is of considerable interest since a high permeate rate is most desirable** in the filtration process. As a result, much research effort has been expended in this area. (emphasis added, Song, page 3389, column 1, 2nd paragraph of the Introduction)

Based on these teachings of Song, the Examiner must explain why one of skill would be motivated to develop a gel layer during an ultrafiltration step to aid in plasmid DNA purification, as claimed here.

The instant application discloses the benefits of purifying plasmid DNA by including a filtration step which involves passing solutions containing plasmid DNA

through an ultrafilter on which a gel layer composed of plasmid DNA has formed. The specification specifically teaches how to form a gel layer. See *e.g.* specification, page 13, line 22 - page 14, line 20. The presence of the gel layer during the ultrafiltration process results in higher yields and greater purity of the plasmid being isolated.

As indicated above by the Court of Appeals for the Federal Circuit, even if an element is inherent in a process, there must be a teaching or suggestion which supports the use of the element in the particular claimed combination. Nothing in Song teaches or suggests that gel layers are beneficial in an ultrafiltration step to aid in plasmid DNA purification. Nothing in Song would motivate one of skill in the art to specifically develop a gel layer during an ultrafiltration step in a plasmid purification method.

In summary, the Examiner has combined references which do not teach or suggest each of the elements of the claimed invention, which teach away from using alkali lysis for pharmaceutical/industrial plasmid DNA purification, which teach away from adding additional purification steps to the steps disclosed in each reference, and which teach away from purifying plasmid DNA by ultrafiltration through a gel layer. None of the references cited by the Examiner teach or suggest the combination of steps disclosed and claimed by the Applicants in the instant application. As discussed above, stating that one of skill in the art would arrive at the claimed invention by combining the cited references in the manner taught by the Examiner is at best taking the Applicants' disclosure "as a blueprint for piecing together the prior art to defeat patentability -- the essence of hindsight". Applicants submit that the Examiner has not established a *prima facie* case of obviousness because the cited references do not teach or suggest all of the elements of the claimed invention, there is no suggestion or motivation to combine the cited references and there is no reasonable expectation that one of skill in the art would arrive at the claimed invention using the teachings of the cited references. Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of claims 1-21 be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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